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Summary and Implications

2-Thiobarbituric acid-reactive substances (TBARS) values of raw pork, and chicken breast and thigh meats did not change during a 7-d storage period. Low free iron content and high ferric ion reducing capacity (FRC) were responsible for the low TBARS values in those meats during storage. TBARS values of raw beef loin, however, significantly increased during 7-d storage because of high free iron content and high lipoxygenase-like activities by ferrylmyoglobin. The TBARS values of cooked meat increased significantly with storage. Heat-stable FRC was detected in all cooked meat and was responsible for the increase of TBARS in cooked meat during storage. The rate of TBARS increases and the amounts of nonheme iron and heat-stable FRC in cooked beef loin were higher than those in cooked pork loin and chicken breast. In spite of lower amounts of nonheme iron and heat-stable FRC, cooked chicken thigh showed similar levels of TBARS to cooked beef loin after 7-d storage because of its high PUFA content. The total amount of PUFA in meat, most of which were present in triglycerides, influenced the development of lipid peroxidation only in the presence of sufficient amounts of free irons in cooked meat.

This indicated that the content of free ionic iron, myoglobin, and ferric ion reducing capacity (FRC) were the primary determinants for the different susceptibility of raw meats to lipid peroxidation. In cooked meat, the contents of free ionic iron and heat-stable FRC played a key role on the development of lipid peroxidation. PUFA was important for lipid oxidation in cooked meat only when sufficient amount of free iron was present.

Introduction

Lipid peroxidation is one of the primary causes of quality deterioration in meat and meat products, and generates compounds that may be detrimental to human health. The conversion of muscle to meat after slaughter destroys the balance between prooxidative and antioxidative factors, resulting in initiation and propagation of lipid peroxidation. The rate of lipid peroxidation in meat depends upon the balance between endogenous and exogenous factors of the meat. The endogenous factors include total lipid content, fatty acid composition of fat, types and amounts of iron present, reducing compounds (e.g., ascorbic acid), natural antioxidants (carnosine, anserine and α-tocopherol), and antioxidant enzymes (catalase, superoxide dismutase, etc). The exogenous factors include oxygen, heating, addition of salts, temperature abuse during handling and distribution, and prolonged storage, etc.

The susceptibility of meat to lipid peroxidation varies among meats from different animal species and muscles from the same animal. Among meats, beef is the most susceptible to lipid peroxidation. Differences in heme pigment content and catalase activity determine the rate of lipid peroxidation in raw meat. It is hypothesized that meats with higher heme pigment content (beef) produce more hydrogen peroxide (H_2O_2) during oxymyoglobin autoxidation than that with less heme pigments. Hydrogen peroxide can react with metmyoglobin to generate ferrylmyoglobin, which can initiate lipid peroxidation. Therefore, catalase activity can be an important determining factor for lipid peroxidation in. In addition to various iron catalysts, differences in fat content, fatty acid composition, endogenous antioxidants such as carnosine and related dipeptides, and antioxidant enzymes may also play important roles for oxidative stability of meat. Reducing compounds such as ascorbic acid can serve as an electron donor in free radical-mediated oxidative processes and plays a critical role in the progress of lipid peroxidation. The role of lipoxygenase in fish tissues as an enzymic initiator of lipid peroxidation has been actively investigated. Lipoxygenase activities are found in various mammalian tissues and can be an important determinant for the oxidative susceptibility of muscle tissues.

Heating accelerates lipid peroxidation and volatile production in meat by disrupting muscle cell structure, inactivating antioxidant enzymes and other antioxidant compounds, and releasing iron from heme pigments. High temperature causes reduction of activation energy for lipid peroxidation and decomposes preformed hydroperoxides to free radicals, which stimulates autoxidation process and off-flavor development further.

The objective of this research was to determine factors influencing oxidative stability of meats from different animal species. Effect of heat treatments on the pro- and antioxidant factors in meats was also examined.

Materials and Methods

Sample preparation

Beef and pork loin muscles from four different animals were purchased from a local packing plant. Loins from each animal were used as a replication. A total of 16 broilers (6-week-old) were slaughtered. Breast and thigh muscles from 4 birds were pooled and used as a replication. Muscles for

each replication were ground separately, and patties (60-g) were prepared after grinding them twice through an 8-mm plate. The patties were individually packaged in oxygen-permeable zipper bags (polyethylene, 4 x 6, 2 mil.). One half of the packaged patties were cooked in bag in a 95 °C water bath to an internal temperature of 75 °C followed by cooling for 2 h at 4 °C. After draining meat juices from the bag, the patties were repackaged. Raw and cooked patties were stored at 4 °C until used. Lipid peroxidation, nonheme iron, reducing compounds, free radical scavenging ability, and lipoxygenase activity of meat samples were determined at 0, 3, and 7 days of storage.

Chemical analyses

The amounts of 2-thiobarbituric acid reactive substances (TBARS) were expressed as mg malondialdehyde (MDA) per kg meat. Nonheme iron content was determined by the ferrozine iron analysis method and expressed as μg nonheme iron per g meat. Total iron content was measured by the wet-ashing method and the ferrozine method.

Free radical scavenging activities of meat were determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. For lipoxygenase activities minced meat (5 g) was homogenized with 15 ml of 50 mM acetate buffer (pH 5.8) using a Polytron for 10 s at top speed, and then centrifuged. The supernatant was filtered through a Whatman No. 1 filter paper and the filtrate was used to determine lipoxygenase activities in meat. Lipoxygenase activity was assessed at 27 °C by the increase of absorbance at 234 nm produced by the formation of conjugated diene from linoleic acid. The reaction mixture was composed of 80 μl sodium linoleate solution (10 mM), 80 μl enzyme solution, and 50 mM acetate buffer (pH 5.8) to a final volume of 1 ml. The results were expressed as units of activity (U) per g meat, and calculated from the molar extinction coefficient of hydroperoxyl linoleic acid (ε = 25,000 $M^{-1}cm^{-1}$). One unit of lipoxygenase activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of hydroperoxide per minute.

Results and Discussion

Lipid peroxidation

The initial (Day 0) TBARS values for all raw meats from different animal species were not different from each other. At Day 3 and Day 7, however, the TBARS values of beef loin were significantly higher than those of pork loin, chicken breast, and chicken thigh, and the rate of TBARS increase among raw meat during storage was the highest in beef loin. The TBARS values of raw pork loin, chicken breast, and chicken thigh did not increase significantly during storage (Figure 1A).

In cooked meat, the TBARS values of all meats gradually increased with storage time (Figure 1B). The TBARS values of cooked beef loin and chicken thigh were approximately 2 times greater than those of pork loin and chicken breast after 7 days of storage.

Total fat content and fatty acid composition

Beef loin and chicken thigh had higher total fat content than pork loin and chicken breast in both raw and cooked meat. The composition of fat is more important than the amount of fat in meat because the susceptibility of muscle lipid to lipid peroxidation depends upon the degree of polyunsaturation in fatty acids. The proportion of PUFA in total fat of chicken breast and thigh was significantly higher than that of pork and beef loin. TG is the most abundant lipid class and most TG is stored in adipose tissues where the amounts of prooxidants such as myoglobin are low. The PUFA content (%) in TG and total fat from chicken breast and thigh were higher than those from pork and beef loin. PL is primarily responsible for rancidity and warmed-over flavor development in raw and cooked meat because the high susceptibility of PL to oxidative changes is attributed not only to its high PUFA content but also its omnipresence in cell membrane. Therefore, differences in the amount of total fat and PUFA content may not be proportionately related to the different rates of lipid peroxidation in raw meats. However, they could be important factors affecting the rate of lipid peroxidation in cooked meat because heat disrupts cell membrane structure to increase the accessibility of prooxidants to PUFA in TG although prooxidants still have easier access to PUFA in cell membrane than that of TG in adipose tissues.

Fatty acid compositions showed that PE was more polyunsaturated than PC. Although statistical differences in the amounts of PUFA in PC and PE of raw and cooked meat from different animal species were observed, those differences did not influence TBARS values (Figure 1). The PUFA contents in PE and PC of raw and cooked beef loin were similar to those of pork loin, chicken breast, and chicken thigh although beef loin contained significantly lower PUFA contents in total fat than chicken breast, chicken thigh and pork loin. Therefore, prooxidant and antioxidant factors other than fatty acid composition should be involved in high TBARS values of raw and cooked beef loin compared with other meats.

Total and nonheme iron

Iron is the most abundant transitional metal in biological systems and can change its oxidation states, reduction potential, and electronic configurations. Iron has been suggested to play important roles in lipid peroxidation as a catalyst and an initiator. Iron can catalyze the production of hydroxyl radical ($^{\bullet}OH$) *in vivo* and *in vitro* via the Fenton reaction. Also, ferrylmyoglobin formed by the interaction of H_2O_2 with metmyoglobin can abstract a hydrogen atom from bisallylic position of PUFA to initiate lipid peroxidation. Iron is distributed in five distinct pools in biological system: transferrin, ferritin, heme pigments, iron-dependent enzymes, and small transit pools of iron chelates

(so called "free" iron), respectively. "Free" iron and/or ferrylmyoglobin are primarily responsible for lipid peroxidation in raw and cooked meat.

As expected, total iron content in raw and cooked meats was the highest for beef loin, followed by chicken thigh, chicken breast, and pork loin. The content of nonheme iron in chicken thigh was higher than that of other meats at Day 0, and changes of nonheme iron content in pork loin, chicken breast and thigh during storage were not observed. However, nonheme iron content in raw beef loin significantly increased with storage time, and the amount at Day 7 was approximately 2 times as high as that at Day 0. The increase of nonheme iron in raw beef loin during storage could be caused by the release of iron from heme pigments, especially myoglobin by H_2O_2, and related to the increase of TBARS values during storage in Figure 1A. Heating increases nonheme iron content in meats from all animal species. Cooked beef loin and chicken thigh had higher nonheme iron content than cooked pork loin and chicken breast, but nonheme iron content did not change during storage in cooked pork loin, chicken breast and chicken thigh as in raw meat. Cooked beef loin showed an increase of nonheme iron with storage time, indicating that nonheme iron in cooked beef loin might be strongly related to the rate of lipid peroxidation in cooked beef loin although beef loin has low PUFA in total fat.

Ferric ion reducing capacity (FRC)

The ability of antioxidant compounds to reduce ferric ion to ferrous ion such as ferric-reducing antioxidant power (FRAP) assay had been used to evaluate the antioxidant activity in meat. Various reducing compounds including ascorbic acid, NAD(P)H, and thiol compounds such as glutathione (GSH) are present in biological cells and may be primarily responsible for ferric ion reducing capacity (FRC) of meat. The amount of reducing compounds in turkey muscles were ~3 mg ascorbic acid equivalent / 100 g fresh meat, and 80% of the reducing compounds were ascorbic acid. Ascorbic acid is an important biological reducing agent, which is able to serve as an electron donor in free radical-mediated oxidative processes.

In raw meat, FRCs of chicken breast and thigh were significantly higher than those of pork and beef loin. The FRCs of raw chicken breast and thigh decreased with storage time while those of raw beef and pork loin did not change. This suggested that significant amounts of FRCs of raw chicken breast and thigh were storage-unstable but most of FRCs in raw beef and pork loin were storage-stable. Also, it is assumed that high FRC and low concentration of nonheme iron are responsible for low TBARS in raw chicken breast and thigh during storage.

Heating reduced the FRCs of chicken breast and thigh more than those of pork and beef loin. Generally, ascorbic acid is readily decomposed by heating, and, thus, most of FRCs present in cooked meat may not be ascorbic acid. Also, the changes in the amounts of FRCs in pork and beef loin before and after cooking were small, indicating that raw pork and beef loin contained only small amounts of unstable reducing agents such as ascorbic acid.

Heat-stable FRC was detected in all cooked meat. Ferric ion can hardly catalyze lipid peroxidation without reducing compounds to reduce it to ferrous iron, which is a major catalyst form of free ionic iron for lipid peroxidation. Reducing agents can act as prooxidants when their amounts are relatively low. Cooking decreased the FRC but increased nonheme iron of meat, resulting in comparatively lower FRC in cooked meat than raw meat. Therefore, heat-stable FRC may be primarily responsible for the regeneration of ferrous ion to increase TBARS in cooked meat during storage. The heat-stable FRC of beef loin was the highest and very stable during storage, indicating that high "heat-stable" FRC and nonheme iron are responsible for the rapid TBARS increase in cooked beef loin during storage. Although heat-stable FRC in cooked chicken thigh was lower than that in cooked beef loin, their TBARS after 7 days of storage were similar. This was attributed to high amounts of PUFA in cooked chicken compared with beef loin. Heating disrupts membrane barriers in meat and facilitates the access of prooxidants to PUFA in adipose tissues.

Free radical scavenging activities

In raw meat, DPPH radical scavenging activities of chicken breast (11.57% and 20.98%) and thigh (13.82% and 28.62%) meat at Day 0 and Day 3 were significantly higher than those in pork and beef loin, and were similar to or greater than those of 500 ppm sesamol (28.02%). Therefore, high amounts of reducing compounds in chicken breast and thigh could be responsible for the regeneration of antioxidant present. In addition, chicken meats are reported to contain more histidine-containing dipeptides such as carnosine and anserine, which have antioxidant activities than beef and pork. The scavenging activity of raw chicken thigh decreased faster than that of raw chicken breast because of higher total and nonheme iron content, higher PUFA content in chicken thigh than in breast. The initial scavenging activities of raw and cooked beef loin were much lower than those of chicken breast and thigh because beef loin contained low concentrations of reducing compounds and low amounts of carnosine and anserine. Raw beef loin showed no consumption of radical scavenging activities during storage although TBARS values and nonheme iron content in raw beef loin increased with storage, indicating that the reactive compounds such as ferrylmyoglobin rather than free radicals such as $^{\bullet}OH$ are more important in raw beef loin than raw chicken and pork loin. In addition, beef loin showed lower levels of reducing compounds, especially ascorbic acid, which can reduce the ferrylmyoglobin. The level and the increase of nonheme iron in cooked beef loin during storage were much greater than those in raw beef loin, indicating that ionic iron is responsible for the development of lipid peroxidation in

cooked beef loin. Although the radical scavenging activities of raw pork loin at Day 0 were similar to that during storage, TBARS did not increase during storage. It seemed that the amount of antioxidants in raw pork loin were large enough to prevent lipid peroxidation. The production of free radicals and ferrylmyoglobin in pork loin were lower than that in beef loin because of low concentrations of nonheme and heme iron. In addition, pork has significantly higher catalase activities than beef and chicken, which can decrease the production of ferrylmyoglobin.

Lipoxygenase-like activities

Lipoxygenase activity is essential for the biosynthesis of eicosanoids from arachidonic acids in cell membrane. Lipoxygenase has been identified in various mammalian tissues including skeletal. Lipoxygenase is capable of direct oxygenation of PUFA even in PL bound to membrane to generate lipid hydroperoxides. Therefore, lipoxygenase can be involved in the initiation of lipid peroxidation of meat.

Raw beef loin and chicken thigh showed higher lipoxygenase-like activities than chicken breast and pork loin. These trends appeared to be highly related to total iron contents, especially heme iron content in meat. Lower heme iron content in chicken breast and pork loin may be responsible for their lower lipoxygenase-like activities. The initial lipoxygenase-like activities in raw chicken thigh were similar to those in raw beef loin, but decreased during storage. The lipoxygenase-like activities in beef loin increased considerably during storage. The differences in the changes of lipoxygenase-like activities during storage between chicken thigh and beef loin could be attributed to the difference in the concentration of reducing compounds, especially ascorbic acid. Because ascorbic acid can reduce ferrylmyoglobin, relatively high lipoxygenase-like activity in beef loin should be attributed to high concentration of myoglobin and low concentration of reducing compounds.

The lipoxygenase-like activities in meat decreased dramatically after cooking, indicating that the enzymes are heat-labile. Ferrylmyoglobin generated by the interaction of metmyoglobin and H_2O_2 and hydroxyl radical by the Fenton reaction can directly abstract a hydrogen atom from PUFA and generate lipid hydroperoxide in a similar manner to lipoxygenase. However, most of these activities were not from $^{\bullet}OH$ generated from the Fenton reaction. Therefore, ferrylmyoglobin should be primarily responsible for the lipoxygenase-like activities measured in this study.

Figure 1. *TBARS values of raw (A) and cooked (B) chicken breast, chicken thigh, pork loin, and beef loin during storage at 4 °C (mg MDA / kg meat).* Means with different letters (a-d) within meats from different animal species are significantly different ($P < 0.05$). Means with different letters (x-z) within a storage period are significantly different ($P < 0.05$). n = 4.